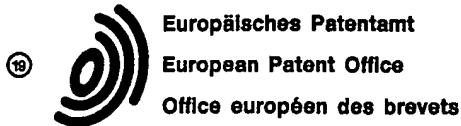


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(54) **Multivesicular liposomes having a biologically active substance encapsulated therein in the presence of a hydrochloride.**

(57) Disclosed are large multivesicular liposomes containing biologically active substances, the multivesicular liposomes having defined size distribution, adjustable average size, adjustable internal chamber size and number, and having substantially higher encapsulation efficiency and substantially slower leakage rate of the biologically active substance than in the previous art. The process comprises dissolving a lipid component in volatile organic solvents, adding an immiscible aqueous component containing a hydrochloride and one or more biologically active substances to be encapsulated, making a water-in-oil emulsion from the two components, immersing the emulsion into a second aqueous component, dividing the emulsion into small solvent spherules which contain even smaller aqueous chambers, and then evaporating the solvents to give an aqueous suspension of multivesicular liposomes encapsulating biologically active substances.

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Description**Multivesicular Liposomes Having a Biologically Active Substance Encapsulated Therein in the Presence of a Hydrochloride****5 Field of the Invention**

The invention relates to synthetic multivesicular lipid vesicles or liposomes encapsulating biologically active substances and processes for their manufacture.

Background of the Invention

10 Multivesicular liposomes are one of the three main types of liposomes, first made by Kim, et. al. (1983, Biochim. Biophys. Acta 782, 339-348), and are uniquely different from the unilamellar (Huang, 1969, Biochemistry 8, 334-352; Kim, et. al. 1981, Biochim. Biophys. Acta 646, 1-10) and multilamellar (Bangham, et. al. 1965, J. Mol. Bio. 13, 238-252), liposomes in that there are multiple non-concentric aqueous chambers within. The prior art describes a number of techniques for producing liposomes, but all of these techniques
15 relate to the production of non-multivesicular liposomes; for example, U.S. Patent No. 4,522,803 to Lenk; 4,310,506 to Baldeschwieler; 4,235,871 to Papahadjopoulos; 4,224,179 to Schneider; 4,078,052 to Papahadjopoulos; 4,394,372 to Taylor; 4,308,166 to Marchetti; 4,485,054 to Mezel; and 4,508,703 to Redziniak, all describe non-multivesicular vesicles. For a comprehensive review of various methods of liposome preparation, refer to Szoka, et. al., 1980, Ann. Rev. Biophys. Bioeng. 9:467-508.

20 The method of Kim, et. al. (1983, Biochim. Biophys. Acta 782,339-348) is the only report that describes multivesicular liposomes, but the encapsulation efficiency of some of the small molecules such as ara-C was relatively low, and the leakage rate of encapsulated molecules in biological fluid was high.

Optimal treatment with many drugs requires maintenance of a drug level for a prolonged period of time. For example, optimal anti-cancer treatment with cell cycle-specific antimetabolites requires maintenance of a
25 cytotoxic drug level for a prolonged period of time. Cytarabine is a highly scheduled-dependent anti-cancer drug. Because this drug kills cells only when they are making DNA, a prolonged exposure at therapeutic concentration of the drug is required for optimal cell kill. Unfortunately, the half-life of Cytarabine after an intravenous (IV) or subcutaneous (SC) dose is very short. To achieve optimal cancer cell kill with a cell cycle phase-specific drug like Cytarabine, two major requirements need to be met: first, the cancer must be
30 exposed to a high concentration of the drug without doing irreversible harm to the host; and second, the tumor must be exposed for a prolonged period of time so that all or most of the cancer cells have attempted to synthesize DNA in the presence of Cytarabine.

Prior to the present invention, the only way of achieving a prolonged plasma cell is through continuous IV or SC infusion, both of which are inconvenient and costly. Therefore, an acceptable slow-release in depot
35 preparation of these drugs is needed. In the past, investigators have attempted to achieve this by chemical modification of the drug molecule to retard metabolism or covalent attachment of a hydrophobic moiety to retard solubilization. Such manipulations have resulted in new toxic effects, Finkelstein, et. al., Cancer Treat Rep 63:1331-1333, 1979, or unacceptable pharmacokinetic or formulation problems, Ho et. al., Cancer Res. 37:1640-1643, 1977.

40 Accordingly, a slow release depot preparation which provides a prolonged and sustained exposure at therapeutic concentration of a biologically-active substance is needed. The present invention is directed to such a preparation.

Summary of the Invention

45 The present invention provides a multivesicular liposome containing a biologically active substance encapsulated in the presence of a hydrochloride which provides a prolonged and sustained exposure at therapeutic concentration of the biologically active substance for optimal results. The present invention also provides methods of making such multivesicular liposomes.

50 The multivesicular liposomes have high encapsulation efficiency, low leakage rate of the encapsulated substance, well defined, reproducible size distribution, spherical shape, adjustable average size that can be easily increased or decreased, adjustable internal chamber size and number.

The process for producing the multivesicular lipid vesicles or liposomes comprises dissolving in one or more organic solvents a lipid component containing at least one neutral lipid and at least one amphipathic lipid with one or more net negative charges, adding into the lipid component an immiscible first aqueous
55 component containing a hydrochloride and one or more substances to be encapsulated, forming a water and oil emulsion from the two immiscible components, transferring and immersing the water and oil emulsion into a second immiscible aqueous component, dispersing the water and oil emulsion to form solvent spherules containing in them multiple droplets of the first aqueous component, and evaporating the organic solvents from the solvent spherules to form the multivesicular liposomes. The use of hydrochlorides, such as
60 hydrochloric acid, is essential for high encapsulation efficiency and for slow leakage rate of encapsulated molecules in biological fluids and *in vivo*. When the hydrochloride is acidic, it is also essential to use a neutralizing agent of low ionic strength to prevent solvent spherules from sticking to each other.

Accordingly, it is an object of the present invention to provide a slow release depot preparation which

provides a prolonged and sustained exposure of a biologically active substance at a therapeutic concentration.

It is a further object of the present invention to provide a method of preparing such a depot preparation.

A further object of the present invention is the provision of a multivesicular liposome having a biologically active substance encapsulated therein and having a prolonged and sustained exposure at a therapeutic concentration.

It is a further object of the present invention is the provision of a multivesicular liposome containing a biologically active substance encapsulated within it in the presence of a hydrochloride which provides a prolonged exposure at therapeutic concentration of the biological active substance for optimal results.

A further object of the present invention is the provision of a method of preparing such a multivesicular liposome.

Other and further objects, features, and advantages of the invention are inherent therein and appear throughout the specification and claims.

Description of Preferred Embodiments

The term "multivesicular liposomes" as used throughout the specification and claims means man-made, microscopic lipid-vesicles consisting of lipid bilayer membranes, enclosing multiple non-concentric aqueous chambers. In contrast, unilamellar liposomes have a single aqueous chamber; and multilamellar liposomes have multiple "onion-skin" type of concentric membranes, in between which are shell-like concentric aqueous compartments.

The term "solvent spherule" as used throughout the specification and claims means a microscopic spheroid droplet of organic solvent, within which is multiple smaller droplets of aqueous solution. The solvent spherules are suspended and totally immersed in a second aqueous solution.

The term "neutral lipid" means oil or fats that have no membrane-forming capability by themselves and lack hydrophilic "head" group.

The term "amphipathic lipids" means those molecules that have a hydrophilic "head" group and hydrophobic "tail" group and have membrane-forming capability.

The term "ionic strength" is defined as: $\text{ionic strength} = 1/2 (M_1Z_1^2 + M_2Z_2^2 + M_3Z_3^2 + \dots)$ where $M_1M_2M_3 \dots$ represent the molar concentrations of various ions in the solution and $Z_1Z_2Z_3 \dots$ are their respective charges.

The term "low ionic strength" is ionic strength less than approximately 0.05, and preferably less than 0.01.

Briefly, "water-in-lipid" emulsion is first made by dissolving amphipathic lipids in a volatile organic solvent for the lipid component, adding to the lipid component an immiscible first aqueous component, the substance to be encapsulated and hydrochloride, and then emulsifying the mixture mechanically. In the emulsion, the water droplets suspend in the organic solvent will form the internal aqueous chambers, and the monolayer of amphipathic lipids lining the aqueous chambers will become one leaflet of the bilayer membrane in the final product. The whole emulsion is then immersed in the second aqueous component containing one or more nonionic osmotic agents and an acid-neutralizing agent of low ionic strength and then agitated either mechanically, by ultrasonic energy, nozzle atomizations or combinations thereof to form solvent spherules suspended in the second aqueous component. The solvent spherules contain multiple aqueous droplets with the substance to be encapsulated dissolved in them. The volatile organic solvent is evaporated from the spherules by passing a stream of gas over the suspension. When the solvent is completely evaporated, the spherules convert into multivesicular liposomes. Representative gases satisfactory for use include nitrogen, helium, argon, oxygen, hydrogen and carbon dioxide.

The use of a hydrochloride is essential for high encapsulation efficiency and for a slow leakage rate of encapsulated molecules in biological fluids and *in vivo*. When the hydrochloride is acidic, it is also essential to use a neutralizing agent of low ionic strength to prevent the solvent spherules from sticking to each other. Hydrochloric acid is preferred but other hydrochlorides which are satisfactory include lysine hydrochloride, histidine hydrochloride and combinations thereof. The amounts of both the hydrochloride and the neutralizing agent used can range from about 0.1mM to about 0.5M concentrations and preferably from about 10mM to about 200mM concentration.

Many different types of volatile hydrophobic solvents such as ethers, hydrocarbons, halogenated hydrocarbons, or Freons may be used as the lipid-phase solvent. For example, diethyl ether, isopropyl and other ethers, chloroform, tetrahydrofuran, halogenated ethers, esters and combinations thereof are satisfactory.

In order to prevent the solvent spherules from sticking to each other and to the vessel wall, at least 1 percent molar ratio of an amphipathic lipid with a net negative charge needs to be included in the spherules, the suspending aqueous solution needs to have a very low ionic strength, and, when the hydrochloride is an acid, a neutralizing agent of low ionic strength is needed to absorb the hydrochloride; otherwise, the solvent spherules coalesce to form a messy scum. One or more nonionic osmotic agents are needed in the suspending aqueous solution to keep the osmotic pressure within and without the liposomes balanced. Various types of lipids can be used to make the multivesicular liposomes, and the only two requirements are that one neutral lipid and one amphipathic lipid with a net negative charge be included. Examples of neutral lipids are triolein, trioctanion, vegetable oil such as soybean oil, lard, beef fat, tocopherol, and combinations thereof. Examples of amphipathic lipids with net negative charge are cardiolipin, the phosphatidylserines,

phosphatidylglycerols, and phosphatidic acids.

The second aqueous components is an aqueous solution containing solutes such as carbohydrates including glucose, sucrose, lactose, and amino acids such as lysine, free-base histadine and combinations thereof.

5 Many and varied biological substances can be incorporated by encapsulation within the multivesicular liposomes. These include drugs, and other kinds of materials, such as DNA, RNA, proteins of various types, protein hormones produced by recombinant DNA technology effective in humans, hematopoietic growth factors, monokines, lymphokines, tumor necrosis factor, inhibin, tumor growth factor alpha and beta, Mullerian inhibitor substance, nerve growth factor, fibroblast growth factor, platelet-derived growth factor, pituitary and
10 hypophyseal hormones including LH and other releasing hormones, calcitonin, proteins that serve as immunogens for vaccination, and DNA and RNA sequences.

The following Table 1 includes a list of representative biologically active substances which can be encapsulated in multivesicular liposomes in the presence of a hydrochloride and which are effective in
15 humans.

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Table 1Antiasthma

metaproterenol
aminophylline
theophylline
terbutaline
Tegretol
ephedrine
isoproterenol
adrenalin
norepinephrine

Antiarrhythmic

propanolol
atenolol
verapamil
captopril
isosorbide

Tranquilizers

chlorpromazine
benzodiazepine
butyrophenones
hydroxyzines
meprobamate
phenothiazines
reserpine
thioxanthines

Cardiac glycosides

digitalis
digitoxin
lanatoside C
digoxin

Hormones

antidiuretic
corticosteroids
testosterone
estrogen
thyroid
growth
ACTH
progesterone
gonadotropin
mineralocorticoid
LH
LHRH
FSH
calcitonin

Steroids

prednisone
triamcinolone
hydrocortisone
dexamethasone
betamethasone
prednisolone

Antihypertensives

apresoline
atenolol

Antidiabetic

Diabenese
insulin

Antihistamines

pyribenzamine
chlorpheniramine
diphenhydramine

Antiparasitic

praziquantel
metronidazole
pentamidine

Anticancer

azathioprine
bleomycin
cyclophosphamide
adriamycin
daunorubicin
vincristine
methotrexate
6-TG
6-MP
vinblastine
VP-16
VM-26
cisplatin
FU

Sedatives & Analgesic

morphine
dilauidid
codeine
codeine-like synthetics
demerol
oxymorphone
phenobarbital
barbiturates

5	<u>Antibiotic</u>	<u>Immunotherapies</u>	<u>Vaccines</u>
	penicillin	interferon	influenza
	tetracycline	interleukin-2	respiratory syncytial
10	erythromycin	monoclonal antibodies	virus
	cephalothin	gamaglobulin	Hemophilus influenza
	imipenem		vaccine
	cefotaxime	<u>Antifungal</u>	
	carbenicillin		<u>Antiviral</u>
15	vancomycin	amphotericin B	acyclovir and deriva-
	gentamycin	myconazole	tives
	tobramycin	muramyl dipeptide	Winthrop-51711
	piperacillin	clotrimazole	ribavirin
	moxalactam		rimantadine/amantadine
20	amoxicillin	<u>Antihypotension</u>	azidothymidine & deriva-
	ampicillin		tives
	cefazolin	dopamine	adenine arabinoside
	cefadroxil	dextroamphetamine	amidine-type protease
	cefoxitin		inhibitors
25	other aminoglycosides		
	<u>Proteins and Glycoproteins</u>		<u>Other</u>
	lymphokines		cell surface receptor
	interleukins - 1, 2, 3, 4, 5, and 6		blockers
30	cytokines		
	GM-CSF		<u>Nucleic Acids & Analogs</u>
	M-CSF		DNA
	G-CSF		RNA
	tumor necrosis factor		methylphosphonates
	inhibin		and analogs
35	tumor growth factor		
	mullerian inhibitors substance		
	nerve growth factor		
	fibroblast growth factor		
	platelet derived growth factor		
40	coagulation factors (e.g. VIII, IX, VII)		
	insulin		
	tissue plasminogen activator		
	histocompatibility antigen		
	oncogene products		
45	myelin basic protein		
	collagen		
	fibronectin		
	laminin		
	other proteins made by recombinant DNA		
50	technology		

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The dosage range appropriate for human use include the range of 1-6000 mg/m to body surface area. The reason that this range is so large is that for some applications, such as subcutaneous administration, the dose

required may be quite small, but for other applications, such as intraperitoneal administration, the dose desired to be used may be absolutely enormous. While doses outside the foregoing dose range may be given, this range encompasses the breadth of use for practically all the biologically active substances.

The multivesicular liposomes may be administered by any desired route; for example, intrathecal, intraperitoneal, subcutaneous, intravenous, intralymphatic, oral and submucosal, under many different kinds of epithelia including the bronchial epithelia, the gastrointestinal epithelia, the urogenital epithelia, and various mucous membranes of the body, and intramuscular.

The following examples represent presently preferred methods of preparing these multivesicular liposomes encapsulating biologically active substances.

EXAMPLE 1

Step 1) In a clean one-dram glass vial (1.4 cm diameter X 4.5 cm height in external dimensions), 9.3 umoles of dioleoyl lecithin, 2.1 umoles of dipalmitoyl phosphatidylglycerol, 15 umoles of cholesterol, 1.8 umoles of triolein and one ml of chloroform were placed (the lipid phase).

Step 2) One ml of aqueous phase, cytosine arabinoside (20 mg./ml) dissolved in 0.136 N hydrochloric acid solution, is added into the above one-dram vial containing lipid phase.

Step 3) For making the water-in-oil emulsion, the vial is sealed and attached to the head of a vortex shaker and shaken at maximum speed for 6 minutes.

Step 4) For making the chloroform spherules suspended in water, half of the emulsion is then each squirted rapidly through a narrow tip Pasteur pipette into one dram vials, each containing 4 percent dextrose in water and 40 mM lysine, free base, and then shaken on the vortex shaker for 3 seconds at half speed to form the chloroform spherules.

Step 5) The chloroform spherule suspensions in the two vials are poured into the bottom of a 250 ml Erlenmeyer flask containing 5 ml of water, glucose (3.5 g/100 ml), and free-base lysine (40 mM) and a stream of nitrogen gas at 7 l/minute is flushed through the flask to slowly evaporate chloroform over 10 - 15 minutes at 37° C. The liposomes are then isolated by centrifugation at 600 X g for 5 minutes.

The average volume-adjusted size of the resulting liposomes (\pm standard deviation of the distribution) was 19.4 ± 6.5 μ m. Percentage of capture was 59 ± 7 percent and capture volume was 36 ± 4 μ l/mg of total lipids used. The addition of hydrochloric had marked influence on the rate of cytosine arabinoside leakage from the multivesicular liposomes incubated in human plasma. Half of the drug leaked out in 12 days hydrochloric acid is added in the example above, whereas half of the drug leaked out in only 12 hours when the hydrochloric acid was omitted in step 2 above. When tested in animals, the addition of hydrochloric again had marked influence on the rate of cytosine arabinoside release; the drug stayed within mouse peritoneal cavity much longer when hydrochloric acid is added during manufacture.

The following example illustrates a scale up of the procedure for making large batches of multivesicular liposomes.

EXAMPLE 2

Step 1) In a stainless-steel homogenizer (Omni-Mixer 17150, Sorval Co., Newtown, CT) add 186 umoles of dioleoyl lecithin, 42 umoles of dipalmitoyl phosphatidylglycerol, 300 umoles of cholesterol, 36 umoles of triolein and 20 ml of chloroform were placed (the lipid phase).

Step 2) Twenty ml of aqueous phase, cytosine arabinoside (20 mg/ml) dissolved in 0.136 N hydrochloric acid solution is added into the above stainless-steel mixer containing lipid phase while swirling.

Step 3) For making the water-in-oil emulsion, the homogenizer is sealed and run at "7" setting for 3 minutes.

Step 4) For making the chloroform spherules suspended in water, half of the emulsion is then each poured (while swirling) into two other stainless-steel homogenizer vessels, each containing 200 ml of 4 percent dextrose in water and 40 mM lysine, free base, and then homogenized on the Omni-Mixer for 3 seconds at "1" setting.

Step 5) The chloroform spherule suspensions in each homogenizer vessels are poured into a flat-bottomed, rectangular steel container, 8 in X 12 in the bottom dimension and a stream of nitrogen gas or air at 14 l/minute was flushed through the flask to slowly evaporate chloroform over 10 - 15 minutes. The liposomes are then isolated by centrifugation at 600 X g for 5 minutes.

The following example illustrates methods of making smaller or larger liposomes.

EXAMPLE 3

To make liposomes smaller than that in Example 1 or 2, the mechanical strength or duration of shaking or homogenization in Step 4 of Example 1 or 2 was increased. To make liposomes larger, the mechanical strength or duration of shaking or homogenization in Step 4 of Example 1 or 2 was decreased.

The following Example 4 illustrates representative methods of making liposomes of various lipid compositions and incorporating various material into liposomes.

Example 4

In Step 1) of Example 1 or 2, other amphipathic lipids such as phosphatidyl chollnes (PC), cardiolupin (CL), dimyristoyl phosphatidylglycerol (DMPG), phosphatidyl ethanolamines (PE), phosphatidyl serines (PS),

dimyristoyl phosphatidic acid (DMPA) in various combinations can be used with similar results. For example, PC/C/CL/TO in 4.5/4.5/1/1 molar ratio; DOPC/C/PS/TO in 4.5/4.5/1/1 molar ratio; PC/C/DPPG/TC in 5/4/1/1 molar ratio; 1 PC/C/PG/TC in 5/4/1/1 molar ratio; PE/C/CL/TO in 4.5/4.5/1/1 molar ratio; PC/C/DMPA/TO in 4.5/4.5/1/1 molar ratio can all be used. To incorporate other biogenetic active materials, simply substituted the cytosine arabinoside with a desired material or combination of materials of Table 1 in Step 2 of Example 1 or 2. All of these provide multivesicular liposomes which are effective in humans and provide prolonged exposure of the biologically-active substance at therapeutic concentration.

Thus, the present invention provides "depot" preparations of wide application and uses in which biologically active substances are encapsulated in relatively large amounts and provide prolonged exposure at therapeutic concentrations of these substances for optimal results which avoid high peaking of dosage, which could be toxic.

The present invention, therefore, is well suited and adapted to attain the ends and objects and has the advantages and features mentioned as well as other inherent therein.

While presently preferred embodiments of the invention have been given for the purpose of disclosure, changes may be made therein which are within the spirit of the invention as defined by the scope of the appended claims.

Claims

1. A process for producing multivesicular lipid vesicles or liposomes comprising the steps of:
 - (a) dissolving a lipid component in one or more organic solvents wherein the said lipid component contains at least one neutral lipid and at least one amphipathic lipid with at least one net negative charge;
 - (b) adding into the said lipid component an immiscible first aqueous component containing a hydrochloride and one or more substances to be encapsulated;
 - (c) forming a water-in-oil emulsion from the two immiscible components;
 - (d) transferring and immersing the water-in-oil emulsion into a second immiscible aqueous component;
 - (e) dispersing the water-in-oil emulsion to form solvent spherules containing multiple droplets of the first aqueous component therein; and
 - (f) evaporating the organic solvents from the solvent spherules to form the multivesicular liposomes.
2. The process according to Claim 1 wherein the lipid component is selected from the group consisting of a phospholipid and an admixture of phospholipids.
3. The process according to Claim 2 wherein the phospholipids are selected from the group consisting of phosphatidylcholine, cardiolipin, phosphatidylethanolamine, sphingomyelin, lysophosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and phosphatidic acid.
4. The process according to Claim 2 wherein at least one of the phospholipids is selected from the group with at least one net negative charge.
5. The process according to Claim 2 wherein the phospholipid is provided in admixture with cholesterol.
6. The process according to Claim 2 wherein the phospholipid is provided in admixture with stearylamine.
7. The process according to Claim 1 wherein a lipophilic biologically active material is provided in admixture with the lipid component.
8. The process according to Claim 1 wherein the neutral lipid is selected from the group consisting of triolein, trioctanoin, vegetable oil, lard, beef fat, tocopherol, and combinations thereof.
9. The process according to Claim 1 wherein, the organic solvent is selected from the group consisting of diethyl ether, isopropyl ether, chloroform, tetrahydrofuran, ethers, hydrocarbons, halogenated hydrocarbons, halogenated ethers, esters, and combinations thereof.
10. The process according to Claim 1 wherein the hydrochloride is selected from the group consisting of hydrochloric acid, lysine hydrochloride, histidine hydrochloride, and combinations thereof.
11. The process according to Claim 1 where the substance is a hydrophilic biologically active material.
12. The process according to Claim 1 wherein the emulsification of the two said components is carried out using a method selected from the group consisting of mechanical agitation, ultrasonic energy, and nozzle atomization.
13. The process according to Claim 12 wherein the liposome's average size and number of the aqueous chambers within them are determined by the type, intensity, and duration of the method selected.
14. The process according to Claim 1 wherein

- the hydrochloride is acidic, and
the second aqueous component contains at least one neutralizing agent.
15. The process according to Claim 14 wherein
the neutralizing agent is selected from the group consisting of free-base lysine and free base histidine
and a combination thereof. 5
16. The process according to Claim 1 wherein
the second aqueous component is of low ionic strength.
17. The process according to Claim 16 wherein
the second aqueous component is an aqueous solution containing solutes selected from the group
consisting of carbohydrates and amino acids. 10
18. The process according to Claim 16 wherein
the second aqueous component is an aqueous solution containing solutes selected from the group
consisting of glucose, sucrose, lactose, free-base lysine, free-base histidine, and combinations thereof.
19. The process according to Claim 1 wherein
the formation of the solvent spherules is carried out using methods selected from the group
consisting of mechanical agitation, ultrasonic energy, nozzle atomization, and combinations thereof. 15
20. The process according to Claim 19 wherein
the liposome's average size is determined by the type, intensity, and duration of the energy used.
21. The process according to Claim 1 wherein
the evaporation of the organic solvent is provided by passing gas over the second aqueous
components. 20
22. The process, of Claim 1 where,
the substance to be encapsulated is selected from the group consisting of the compositions of
Table 1 and combinations thereof.
23. A multivesicular liposome containing a biologically active substance encapsulated in the presence of
a hydrochloride. 25
24. A multivesicular liposome containing a biologically active substance encapsulated in the presence of
an acid hydrochloride and neutralized with a neutralizing agent.
25. The multivesicular liposome of Claims 23 or 24 where,
the biologically active substance is selected from the group consisting of the compositions of Table 1
and combinations thereof. 30

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